

**WHAT IS CLAIMED IS:**

1           1.    A reaction mixture for producing a product saccharide, wherein the  
2 reaction mixture comprises an acceptor saccharide and a first type of plant or microorganism  
3 cell that produces: a) a nucleotide sugar, and b) a first recombinant glycosyltransferase that  
4 catalyzes the transfer of a sugar from the nucleotide sugar to the acceptor saccharide to form  
5 the product saccharide.

1           2.    The reaction mixture of claim 1, wherein the cells are selected from one  
2 or more of the group consisting of bacterial cells, yeast cells, fungal cells, and plant cells.

1           3.    The reaction mixture of claim 1, wherein the cells are permeabilized or  
2 otherwise disrupted.

1           4.    The reaction mixture of claim 1, wherein the glycosyltransferase is a  
2 fucosyltransferase and the nucleotide sugar is GDP-fucose.

1           5.    The reaction mixture of claim 1, wherein the glycosyltransferase is a  
2 sialyltransferase and the nucleotide sugar is CMP-sialic acid

1           6.    The reaction mixture of claim 1, wherein nucleotide sugar is selected  
2 from the group consisting of UDP-Gal, UDP-Glc, UDP-Glucuronic acid, UDP-GalNAc,  
3 UDP-Galacturonic acid, GDP-mannose.

1           7.    The reaction mixture of claim 1, wherein the first type of cell produces  
2 the nucleotide sugar at an elevated level compared to a wild-type cell.

1           8.    The reaction mixture of claim 7, wherein the elevated level of the  
2 nucleotide sugar results from a deficiency in the ability of the cell to incorporate the  
3 nucleotide sugar into a polysaccharide normally produced by the cell.

1                   9.    The reaction mixture of claim 7, wherein the elevated level of the  
2   nucleotide sugar is at least 10% higher than the level of the nucleotide sugar produced by the  
3   wild-type cell.

1                   10.   The reaction mixture of claim 9, wherein the elevated level of the  
2   nucleotide sugar is at least 25% higher than the level of the nucleotide sugar produced by the  
3   wild-type cell.

1                   11.   The reaction mixture of claim 1, wherein the nucleotide sugar is  
2   synthesized by an enzymatic pathway that includes one or more enzymes that are expressed  
3   from heterologous genes.

1                   12.   The reaction mixture of claim 11, wherein the recombinant  
2   glycosyltransferase is a sialyltransferase, the nucleotide sugar is CMP-sialic acid and the  
3   heterologous gene encodes CMP-sialic acid synthetase.

1                   13.   The reaction mixture of claim 12, wherein the acceptor saccharide is  
2   lactose and the product saccharide is sialyllactose.

1                   14.   The reaction mixture of claim 11, wherein the recombinant  
2   glycosyltransferase is a  $\beta$ 1,4-GalNAc transferase and the nucleotide sugar is UDP-GalNAc.

1                   15.   The reaction mixture of claim 14, wherein the acceptor is lactose and  
2   the product saccharide is  $\beta$ 1,4-GalNAc-lactose.

1                   16.   The reaction mixture of claim 11, wherein the recombinant  
2   glycosyltransferase is a galactosyltransferase and the nucleotide sugar is UDP-Gal.

1                   17.   The reaction mixture of claim 16, wherein the galactosyltransferase is  
2   an  $\alpha$ 1,3-galactosyltransferase and the product saccharide contains a terminal  $\alpha$ 1,3-linked  
3   galactose residue.

1           18. The reaction mixture of claim 11, wherein the enzymatic pathway  
2 comprises a full or partial sugar nucleotide regeneration cycle.

1           19. The reaction mixture of claim 18, wherein the nucleotide sugar is UDP-  
2 GalNAc and the sugar nucleotide regeneration cycle comprises a set of enzymes selected  
3 from the group consisting of:

4                   UDP-GalNAc epimerase, UDP-GlcNAc pyrophosphorylase, GlcNAc-1-  
5 kinase, polyphosphate kinase and pyruvate kinase; and

6                   UDP-GalNAc pyrophosphorylase, GlcNAc-1-kinase, polyphosphate  
7 kinase and pyruvate kinase.

1           20. The reaction mixture of claim 19, wherein the reaction mixture further  
2 comprises a second cell type that produces a nucleotide that is used as a substrate for the  
3 sugar nucleotide regeneration cycle.

1           21. The reaction mixture of claim 20, wherein the second cell type  
2 comprises an exogenous gene that encodes a nucleotide synthetase polypeptide that catalyzes  
3 the synthesis of the nucleotide.

1           22. The reaction mixture of claim 21, wherein the first cell type comprises  
2 exogenous genes that encode a) a fusion protein that comprises a polypeptide having 3'-  
3 sialyltransferase activity and a polypeptide that has CMP-sialic acid synthetase activity; and  
4 b) enzymes that catalyze the synthesis of sialic acid from GlcNAc;  
5                   and the second cell type comprises an exogenous gene that encodes  
6 CMP-synthetase.

1           23. The reaction mixture of claim 21, wherein the first cell type is *E. coli*  
2 and the second cell type is yeast or *Corynebacterium*.

1                   24. The reaction mixture of claim 1, wherein the first type of cell produces a  
2 second recombinant glycosyltransferase that catalyzes the transfer of a sugar from the  
3 nucleotide sugar to the product saccharide to form a further glycosylated product saccharide.

1                   25. The reaction mixture of claim 24, wherein the nucleotide sugar is UDP-  
2 Gal, the first recombinant glycosyltransferase is an  $\beta$ 1,4-galactosyltransferase and the second  
3 recombinant glycosyltransferase is an  $\alpha$ 1,3-galactosyltransferase.

1                   26. The reaction mixture of claim 25, wherein the acceptor saccharide is  
2 Glc(R) $\beta$ -O-R<sup>1</sup>, wherein R<sup>1</sup> is -(CH<sub>2</sub>)<sub>n</sub>-COX; X is selected from the group consisting of OH,  
3 OR<sup>2</sup>, -NHNH<sub>2</sub>, R is OH or NAc; R<sup>2</sup> is a hydrogen, a saccharide, an oligosaccharide or an  
4 aglycon group having at least one carbon atom, and n is an integer from 2 to 18.

1                   27. The reaction mixture of claim 25, wherein the UDP-Gal is generated by  
2 enzymes that are expressed from exogenous genes that encode UDP-Gal 4' epimerase and  
3 UDP-Glc pyrophosphorylase.

1                   28. The reaction mixture of claim 1, wherein the cell further comprises: a)  
2 an enzymatic system for producing at least a second nucleotide sugar, and b) at least a  
3 second recombinant glycosyltransferase that catalyzes transfer of a sugar from the second  
4 nucleotide sugar to the product sugar.

1                   29. The reaction mixture of claim 28, wherein:  
2 the first recombinant glycosyltransferase is a GlcNAc transferase and  
3 the first nucleotide sugar is UDP-GlcNAc; and  
4 the second recombinant glycosyltransferase is a galactosyltransferase  
5 and the second nucleotide sugar is UDP-galactose.

1                   30. The reaction mixture of claim 29, wherein the reaction mixture forms  
2 lacto-N-neotetraose (LNnT).

1                   31. The reaction mixture of claim 1, wherein the reaction mixture also  
2 comprises at least a second type of cell that produces a) a second nucleotide sugar, and b) a  
3 second recombinant glycosyltransferase that catalyzes the transfer of the sugar from the  
4 second nucleotide sugar to the product saccharide.

1                   32. The reaction mixture of claim 31, wherein the first glycosyltransferase  
2 is a galactosyltransferase and the second glycosyltransferase is a GalNAc transferase.

1                   33. The reaction mixture of claim 31, wherein:  
2                   the first cell type comprises a recombinant  $\beta$ 1,4-GalNAc transferase, a  
3 recombinant  $\beta$ 1,4-Gal transferase, UDP-GalNAc and UDP-Gal; and  
4                   the second cell type comprises a recombinant  $\alpha$ 2,3-sialyltransferase and  
5 CMP-sialic acid.

1                   34. The reaction mixture of claim 33, wherein the CMP-sialic acid is  
2 produced from CTP and GlcNAc by an enzymatic system in the second cell type that  
3 includes recombinant enzymes CMP-sialic acid synthetase, GlcNAc epimerase, NeuAc  
4 aldolase, and CMP-synthetase.

1                   35. The reaction mixture of claim 33, wherein the acceptor saccharide is  
2 lactosylceramide or lyso-lactosylceramide and the product saccharide is ganglioside GM<sub>2</sub>.

1                   36. The reaction mixture of claim 33, wherein the second cell type further  
2 comprises a recombinant  $\alpha$ 2,8-sialyltransferase.

1                   37. The reaction mixture of claim 36, wherein the acceptor is  
2 lactosylceramide or lyso-lactosylceramide and the product saccharide is GD<sub>2</sub>.

1                   38. The reaction mixture of claim 1, wherein the reaction mixture also  
2 comprises a second type of cell that produces a nucleotide from which is synthesized the  
3 nucleotide sugar produced by the first type of cell.

1                   39. The reaction mixture of claim 38, wherein nucleotide produced by the  
2 second cell type and the corresponding nucleotide sugar are selected from the group  
3 consisting of:

4                   UTP: UDP-Gal, UDP-GalNAc, UDP-GlcNAc, UDP-Glc, UDP-  
5 glucuronic acid, or UDP-galacturonic acid;

6                   GTP: GDP-Fuc; and

7                   CTP: CMP-sialic acid.

1                   40. A cell that produces a product saccharide, wherein the cell comprises:

2                   a) a recombinant gene that encodes a glycosyltransferase;

3                   b) an enzymatic system for forming a nucleotide sugar that is a  
4 substrate for the glycosyltransferase; and

5                   c) an exogenous saccharide acceptor moiety;

6                   wherein the glycosyltransferase catalyzes the transfer of a sugar from  
7 the nucleotide sugar to the acceptor moiety to produce the product saccharide.

1                   41. The cell of claim 40, wherein the enzymatic system for forming a  
2 nucleotide sugar comprises cycle enzymes for regenerating the nucleotide sugar.

1                   42. The cell of claim 40, wherein the recombinant gene that encodes a  
2 glycosyltransferase is a heterologous gene.

1                   43. The cell of claim 40, wherein the cell forms the nucleotide sugar at an  
2 elevated level compared to a wild-type cell.

1                   44. The cell of claim 43, wherein the elevated level of nucleotide sugar  
2 results from a deficiency in the ability of the cell to incorporate the nucleotide sugar into a  
3 polysaccharide normally produced by the cell.

1                   45. The cell of claim 44, wherein the deficiency is due to a reduced level of  
2 a polysaccharide glycosyltransferase activity.

1                   46. The cell of claim 40, wherein the product saccharide is produced at a  
2 concentration of at least about 1 mM.

1                   47. The cell of claim 40, wherein the enzymatic system for forming a  
2 nucleotide sugar comprises an enzyme encoded by a heterologous gene.

1                   48. The cell of claim 47, wherein the enzyme encoded by the heterologous  
2 gene is one or more of:

3                               a GDP-mannose dehydratase, a GDP-mannose 3,5-epimerase, and a  
4 GDP-mannose 4-reductase;  
5                               a UDP-galactose 4' epimerase;  
6                               a UDP-GalNAc 4' epimerase;  
7                               a CMP-sialic acid synthetase;  
8                               a pyrophosphorylase selected from the group consisting of a UDP-Glc  
9 pyrophosphorylase, a UDP-Gal pyrophosphorylase, a UDP-GalNAc pyrophosphorylase, a  
10 GDP-mannose pyrophosphorylase, and a UDP-GlcNAc pyrophosphorylase;  
11                               a kinase selected from the group consisting of myokinase, pyruvate  
12 kinase, acetyl kinase, creatine kinase; and  
13                               pyruvate decarboxylase.

1                   49. The cell of claim 48, wherein the nucleotide sugar is GDP-fucose.

1                   50. A cell that produces a sulfated polysaccharide, the cell comprising:

2 a heterologous gene that encodes a sulfotransferase; and  
3 an enzymatic system that produces PAPS.

1 51. The cell of claim 50, wherein the sulfated polysaccharide is selected  
2 from the group consisting of heparin sulfate and carragenin.

1 52. The cell of claim 50, wherein the enzymatic system that produces PAPS  
2 comprises one or more enzymes that are expressed from exogenous genes.

1 53. A method of producing a product saccharide, the method comprising  
2 contacting a microorganism or plant cell with an acceptor saccharide, wherein the cell  
3 comprises:  
4 a) an enzymatic system for forming a nucleotide sugar; and  
5 b) a recombinant glycosyltransferase which catalyzes the transfer of a  
6 sugar from the nucleotide sugar to the acceptor saccharide to produce the product saccharide.

1 54. The method of claim 53, wherein the glycosyltransferase is encoded by  
2 a heterologous gene.

1 55. The method of claim 53, wherein the glycosyltransferase is encoded by  
2 a gene that is endogenous to the cell and is produced by the cell at an elevated level  
3 compared to a wild-type cell.

1 56. The method of claim 53, wherein the product saccharide is produced at  
2 a concentration of at least about 1 mM.

1 57. The method of claim 53, wherein the cell is permeabilized.

1 58. The method of claim 53, wherein the cell is an intact cell.

1 59. The method of claim 53, wherein the enzymatic system for forming a  
2 nucleotide sugar comprises an enzyme that is encoded by a heterologous gene.



1                   60. The method of claim 59, wherein the enzyme encoded by the  
2 heterologous gene is one or more of:  
3                   a GDP-mannose dehydratase, a GDP-4-keto-6-deoxy-D-mannose 3,5-  
4 epimerase, and a GDP-4-keto-6-deoxy-L-glucose 4-reductase;  
5                   a UDP-galactose 4' epimerase;  
6                   a UDP-GalNAc 4' epimerase;  
7                   a CMP-sialic acid synthetase;  
8                   a pyrophosphorylase selected from the group consisting of a UDP-Glc  
9 pyrophosphorylase, a UDP-Gal pyrophosphorylase, a UDP-GalNAc pyrophosphorylase, a  
10 GDP-mannose pyrophosphorylase, and a UDP-GlcNAc pyrophosphorylase; a kinase  
11 selected from the group consisting of myokinase, pyruvate kinase, acetyl kinase, creatine  
12 kinase; and  
13                   pyruvate decarboxylase.

1                   61. The method of claim 59, wherein the enzyme for forming a nucleotide  
2 sugar and the glycosyltransferase are expressed as a fusion protein.

1                   62. The method of claim 61, wherein the fusion protein comprises a CMP-  
2 sialic acid synthetase activity and a sialyltransferase activity.

1                   63. The method of claim 61, wherein the fusion protein comprises a  
2 galactosyltransferase activity and a UDP-Gal 4' epimerase activity.

1                   64. The method of claim 61, wherein the fusion protein comprises a  
2 GalNAc transferase activity and a UDP-GlcNAc 4' epimerase activity.

1                   65. The method of claim 53, wherein the nucleotide sugar is GDP-fucose  
2 and the glycosyltransferase is a fucosyltransferase.

1                   **66.** The method of claim **53**, wherein the cell forms the nucleotide sugar at  
2 an elevated level compared to a wild-type cell.

1                   **67.** The method of claim **66**, wherein the elevated level of nucleotide sugar  
2 results from a deficiency in the ability of the cell to incorporate the nucleotide sugar into a  
3 polysaccharide normally produced by the cell.

1                   **68.** The method of claim **67**, wherein the deficiency is due to a reduced  
2 level of a polysaccharide glycosyltransferase activity.

1                   **69.** The method of claim **53**, wherein the cell/nucleotide sugar are selected  
2 from the group consisting of:

3                   *Azotobacter vinelandii*/GDP-Man;  
4                   *Pseudomonas sp.*/UDP-Glc and GDP-Man;  
5                   *Rhizobium sp.*/UDP-Glc, UDP-Gal, GDP-Man;  
6                   *Erwinia sp.*/UDP-Gal, UDP-Glc;  
7                   *Escherichia sp.*/UDP-GlcNAc, UDP-Gal, CMP-NeuAc, GDP-Fuc;  
8                   *Klebsiella sp.*/UDP-Gal, UDP-GlcNAc, UDP-Glc, UDP-GlcNAc;  
9                   *Hansenula jadinii*/ GDP-Man, GDP-Fuc;  
10                  *Candida famata*/UDP-Glc, UDP-Gal, UDP-GlcNAc;  
11                  *Saccharomyces cerevisiae*/UDP-Glc, UDP-Gal, GDP-Man, GDP-  
12 GlcNAc; and  
13                  *X. campesti*/UDP-Glc, GDP-Man.

1                   **70.** The method of claim **53**, wherein the cell is *Azotobacter vinelandii*, the  
2 nucleotide sugar is GDP-mannose, the acceptor saccharide is lactose, the glycosyltransferase  
3 is mannosyl transferase, and the product saccharide is mannosyl lactose.

